

The University of Chicago

RELATIONSHIP BETWEEN OXYGEN
CONSUMPTION AND NITROGEN
METABOLISM

IV. EXPERIMENTS ON ANIMALS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1934

By

CLARENCE WILLIAM BALDRIDGE

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IV. EXPERIMENTS ON ANIMALS

C. W. BALDRIDGE, M.D.

IOWA CITY

Variations in the rate of oxygen consumption are met with in numerous conditions in which no disturbance in thyroid function has been recognized. Prominent among such conditions are the hemato-poietic diseases. In a series of investigations I have attempted to evaluate some of the abnormal physiologic processes in the blood dyscrasias in terms of their effect on the oxidative processes of the organisms as a whole. Studies have been made on patients with pernicious anemia, leukemia and polycythemia vera, as well as on dogs with acute hemorrhagic anemia, hemolytic anemia, induced polycythemia and phlorhizin diabetes.

The previous communications concerning pernicious anemia, leukemia and polycythemia vera will be briefly summarized. The reader is referred to the original publications for details and bibliographies.

STUDIES ON MAN

Pernicious Anemia.—The object of the studies of pernicious anemia, which were carried out by my associates and me was to determine the effect of rapid erythropoiesis on the total oxygen consumption. Ten patients with severe pernicious anemia were studied, and part of the data have been published.¹ The pertinent information derived from these studies may be summarized as follows:

1. In eight of the ten patients the total oxygen consumption decreased during the period of most rapid erythropoiesis. In the other two patients there was no significant change in the gaseous metabolism. In the eight cases the total decrease in basal metabolic rate varied from 50 to 15 per cent. In patients who were without fever and for whom the control periods were sufficiently long the decrease was found to be between 15 and 20 per cent. This drop occurred regardless of whether the control level was above or below the calculated normal.

From the Department of Physiology, University of Chicago.

1. Baldrige, C. W., and Barer, A.: Studies on the Relationship Between Oxygen Consumption and Nitrogen Metabolism: I. In Pernicious Anemia, *J. Clin. Investigation* **10**:529, 1931.

2. During the period of rapid erythropoiesis and decreased oxygen consumption there was moderate storage of nitrogen in all of the four cases in which nitrogen balances were determined.

3. One large dose of liver extract per week by mouth caused the same gradual decrease in oxygen consumption which occurred with smaller daily doses.

4. In nearly every instance the total oxygen consumption gradually decreased throughout the reticulocytic crisis.

5. It is obvious that the formation of blood greatly exceeded the destruction of blood during the period of decreasing oxygen consumption.

Leukemia.—Chronic leukemia under treatment by irradiation affords a circumstance in which the destruction of blood temporarily exceeds the formation of blood. Nine patients with chronic leukemia were studied before and after irradiation, and the data have been published.² The reader is referred to this publication for details of the metabolic studies and for a discussion of the existing theories as to the cause of the increased gaseous metabolism in leukemia.

The results of our investigations on leukemic patients may be summarized as follows:

Following a large dose of roentgen irradiation there is an abrupt increase in the excretion of nitrogen. The nitrogen balance becomes negative, owing to decreased ingestion and increased endogenous nitrogen catabolism. The total oxygen consumption increases within six hours after irradiation, and the increase may last for four days or may disappear within twenty-four hours. The magnitude and duration of the increase in gaseous metabolism seem to depend on the degree and duration of protein catabolism occasioned by the irradiation.

After the preliminary increase in the excretion of nitrogen which follows irradiation, the urinary nitrogen gradually decreases, often to a level below that of the control period. During this period of lowered catabolism of protein the nitrogen balance becomes positive, and the gaseous metabolism decreases.

Polycythemia Vera.—In polycythemia vera, the destruction of blood can be made to exceed the formation of blood by the administration of phenylhydrazine. Two patients with polycythemia vera were studied during intoxication by phenylhydrazine, and the data have been reported.³

2. Baldrige, C. W., and Barer, A.: Relationship Between Oxygen Consumption and Nitrogen Metabolism: II. In *Leukemia*, Arch. Int. Med. **51**:589 (April) 1933.

3. Barer, A.; Paul, W. D., and Baldrige, C. W.: Studies on the Relationship Between Oxygen Consumption and Nitrogen Metabolism: III. In *Polycythemia Vera*, J. Clin. Investigation **13**:15 (Jan.) 1934.

The results of our studies of leukemia and polycythemia vera were quite similar so far as the relationship between nitrogen catabolism and oxygen consumption was concerned. Phenylhydrazine produced a marked decrease in the number of erythrocytes, a negative nitrogen balance, due largely to the increased catabolism of protein, and an increase in the basal metabolic rate. No attempt has as yet been made to establish a quantitative relationship between the loss of endogenous nitrogen and the increase in oxygen consumption by the body, but the destruction of nonnucleated erythrocytes by phenylhydrazine seems to require about as much oxygen per gram of nitrogen as does the destruction of nucleated leukemic cells by irradiation.

ADDITIONAL STUDIES ON ANIMALS

The oxygen consumption of the dogs was measured by the Benedict closed circuit apparatus with attachments described by Kunde.⁴ Four well trained dogs were used. Before control periods were begun, tests were made daily for periods of ten, thirteen and twenty-eight days, respectively, on three previously trained dogs. Dog 4 had not previously been trained, so that a control period was not begun until the oxygen consumption was fairly constant and the average for several days was below the calculated normal consumption. Tests were made at about the same time each day, at least twenty hours after food was taken and after a preliminary period of rest of at least twenty minutes. The tests were run for twenty minutes, and calculations were based on the last fifteen minutes of this period. Determinations were made daily without exception over a period of about eight months. On a few occasions when extraneous influences of one kind or another caused the dogs to move, the tests were repeated. Seven times the second test was unsatisfactory, and though it was recorded, it was not used in the calculations of averages. Two other determinations were unsatisfactory because the dogs obtained food by mistake. Thus, of a total of eight hundred determinations, nine were considered to be unsatisfactory. One dog suffered a short febrile illness after bleeding, which invalidated the results of one experiment (dog 3, chart 4).

According to the work of Kunde and Steinhaus,⁵ the average basal metabolism of normal dogs is 771.2 calories per square meter each twenty-four hours. This figure has been used as the normal, and the surface area has been calculated from Meeh's formula.⁵ As no attempt has been made to compare the metabolism of one animal with that of another, there is no need to discuss the shortcomings of the Meeh formula for the determination of the surface area of the dog. All of the temperatures were taken rectally.

Hemorrhagic anemia was produced by bleeding the dogs with a needle from the jugular or femoral veins. About 25 cc. of blood per kilogram of body weight was removed on each of two successive days. The blood was removed just after the determination of the basal metabolic rate on the day indicated.

Transfusions were done by the indirect method, the blood being given by means of gravity. Fifty cubic centimeters of 2.5 per cent sodium citrate solution was used

4. Kunde, M. M.: The After Effects of Prolonged Fasting on the Basal Metabolic Rate, *J. Metab. Research* **3**:399, 1923.

5. Kunde, M. M., and Steinhaus, A. H.: Studies on Metabolism: IV. The Basal Metabolic Rate of Normal Dogs, *Am. J. Physiol.* **78**:127 (Sept.) 1926.

as an anticoagulant for each transfusion. About 50 cc. of whole blood per kilogram of body weight was given in experiments 5, 6 and 7. In experiment 8 *B*, dog 1 died the day after receiving 375 cc. of whole blood. The blood given in experiments 5, 6 and 7 was found to be compatible by cross-matching. The blood given to dog 1 in experiment 8 *B* was from the donor that had been used in the three transfusions for dog 1 in experiment 5, but cross-matching was not done before the last and fatal transfusion.

The citrated plasma which was given intravenously in experiments 8 *A* and 9 *B* was obtained from the donors from which these recipients had previously received transfusions of whole blood. In each instance the whole blood had been diluted with 50 cc. of 2.5 per cent sodium citrate, and the citrated plasma obtained by centrifugation.

In experiments 9 *A*, 10 *A* and *B* and 11 *A* the corpuscles and plasma were separated by centrifugation, and the corpuscles were hemolyzed by the addition of distilled water.

The charts for the experiments with phlorhizin are almost self-explanatory. One gram of phlorhizin was given in each instance. Merck's phlorhizin was recrystallized from alcohol as suggested by Lusk.⁶ The recrystallized phlorhizin was dried and suspended in sterile olive oil for subcutaneous injection. The loss of nitrogen through the feces and hair was not determined in these animals.

The diets were as follows:

Dogs 1 and 2, from June 22 to July 13, 1931, were given 250 Gm. of chopped beef lung, 225 Gm. of bread, 250 cc. of milk and about 20 Gm. of bone meal daily in one feeding. From July 14 to October 9, 200 Gm. of chopped beef lung was given; the other constituents were unchanged. From October 10 until the death of dog 1 on December 2 and until the beginning of the phlorhizin experiment in dog 2 on December 17, the chopped beef lung was reduced to 150 Gm. daily, with no other change in the diet.

Dog 3 was given chopped beef lung as follows: from June 22 to July 13, 1931, 300 Gm. daily; from July 14 to October 9, 250 Gm. daily and from October 10 to Jan. 9, 1932, 200 Gm. daily. The amounts of bread (225 Gm.), milk (250 cc.) and bone meal (about 20 Gm.) remained constant from June 22, 1931, to Jan. 9, 1932.

Dog 4 was obtained on July 21, 1931; his diet corresponded with that of dogs 1 and 2 until the beginning of the experiment with phlorhizin on December 24.

Liver extract, when given, was given in addition to the regular diet. Liver extract with iron (equivalent to 300 Gm. of whole liver) was given daily for twenty days to each of the dogs that were bled. In dog 3 it was begun immediately after bleeding while in dogs 1 and 2 it was started ten days after bleeding.

Several determinations were made in addition to the metabolic studies. The weight, pulse rate, respiratory rate, temperature, hemoglobin (Sahli), hematocrit value and erythrocyte and leukocyte counts were determined daily. For these determinations 1 cc. of blood was withdrawn from a vein and added to a few small crystals of neutral potassium oxalate. Care was taken to prevent settling of the corpuscles.

RESULTS

Acute Hemorrhagic Anemia.—After control periods of twelve, twelve and fifteen days, respectively, dogs 1, 2 and 3 were bled about

6. Lusk, G.: The Science of Nutrition, Philadelphia, W. B. Saunders Company, 1928, p. 626.

25 cc. per kilogram of body weight on each of two successive days (charts 1, 2, 3 and 4). The dogs had been in the laboratory for many months and had been on an adequate diet, so that the blood counts of the controls were higher than the average for recently impounded dogs. After bleeding there was a gradual restitution of hemoglobin and erythrocytes in each instance. The previous normal level was reached in about twenty days, while at the end of thirty days after bleeding

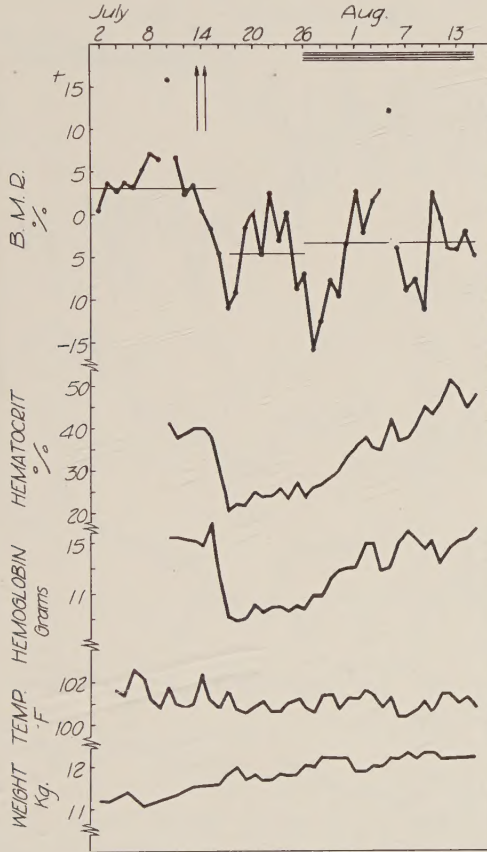


Chart 1 (dog 1).—Two hundred and seventy cubic centimeters of blood was drawn on July 13, and 270 cc. on July 14, 1931, as indicated by the arrows. The dog weighed 11.5 Kg., and the blood volume of all of the dogs was estimated to be 100 cc. per kilogram of body weight. The control period was twelve days, but on one day the metabolism test was unsatisfactory. Liver extract with iron (equivalent to 300 Gm. of whole liver per day) was given for twenty days beginning ten days after bleeding, as indicated by the parallel lines in the upper right hand corner. Here, as in all of the subsequent charts, the control average and the averages for ten-day periods during the experiment are indicated by horizontal lines through the curve of the basal metabolic rate.

there was a distinct and permanent overcorrection in the hemoglobin and the number of erythrocytes. During the first ten days of this period of rapid formation of blood the average oxygen consumption was found to be 7.1, 7.9 and 9.3 per cent, respectively, below the control averages. The basal metabolic rates continued low throughout the entire period of blood regeneration, during which all of the dogs gained in weight (0.4, 1 and 1.2 Kg., respectively). Nitrogen balances were not deter-

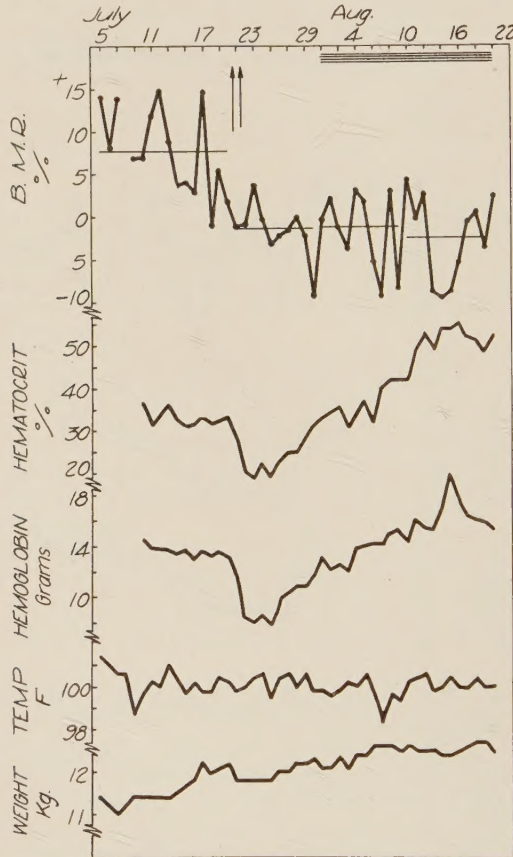


Chart 2 (dog 2).—Two hundred and seventy cubic centimeters of blood was drawn on July 20, and 285 cc. on July 21, 1931. Liver extract was given as in the previous instance (dog 1).

mined, but it is assumed for obvious reasons that the animals were storing nitrogen as well as carbon during the period of recovery.

Dog 3 was bled again at a later date, but an acute febrile illness with vomiting, anorexia and loss of weight followed immediately after the bleeding, so that the results of the experiment were completely invalidated.

The literature on the subject under consideration, though not extensive, is in direct contrast to these results. Eberstadt⁷ found that the gaseous metabolism in rabbits with hemorrhagic anemia was increased, and Rolly⁸ reported a similar rise in the oxygen consumption of dogs after bleeding. Logical objections to the work of these investigators

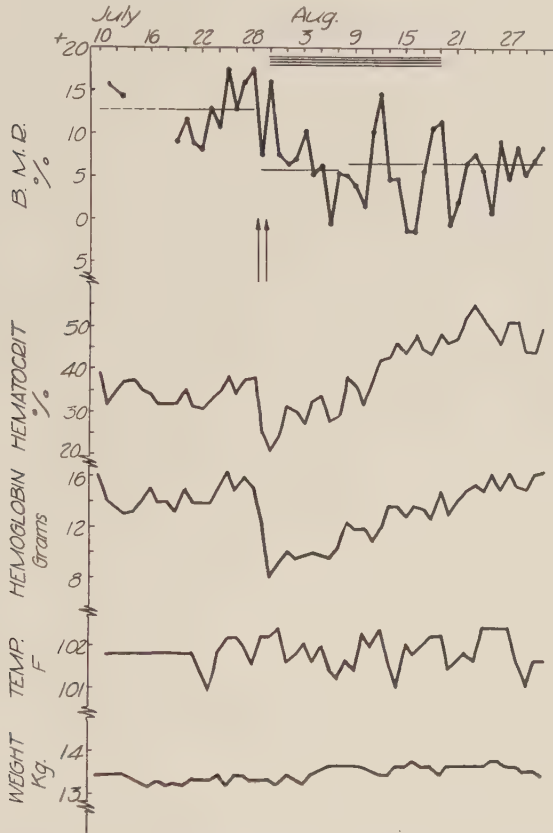


Chart 3 (dog 3).—Three hundred and twenty-five cubic centimeters of blood was drawn on July 28, and 280 cc. on July 29, 1931. Liver extract was given for twenty days, starting immediately after bleeding.

can be based on the fact that determinations on controls were few and on the fact that there was no evidence that the animals had been

7. Eberstadt, F.: Ueber den Einfluss chronischer experimenteller Anämien auf den respiratorischen Gaswechsel, *Arch. f. exper. Path. u. Pharmacol.* **71**:329, 1913.

8. Rolly, F.: Ueber den respiratorischen Gaswechsel bei chronisch anämischen Zuständen, *Deutsches Arch. f. klin. Med.* **114**:605, 1914.

trained. Hawk and Gies⁹ found a negative nitrogen balance in dogs after severe hemorrhage. However, the blood was let under ether anesthesia, and the arteries were cannulated and tied off after the blood was obtained. The femoral artery or some of its branches were used and were permanently occluded. Some slight increase in the urinary

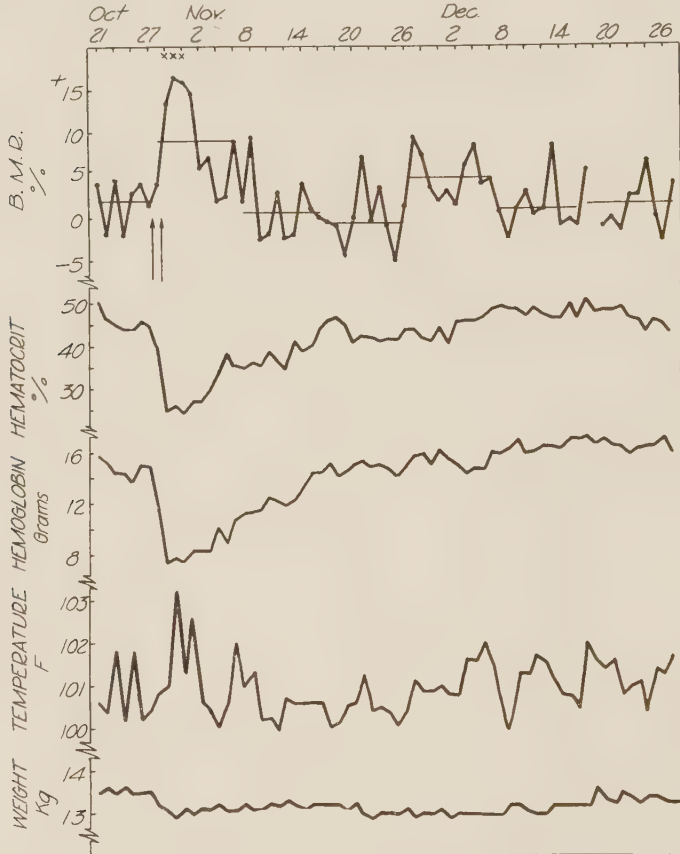


Chart 4 (dog 3).—Three hundred and eighty cubic centimeters of blood was drawn on October 27, and 330 cc. on October 28. The dog was acutely ill with fever, vomiting and anorexia for three days after the last bleeding. There was a loss of about 0.5 Kg. in weight. This result may be similar to those obtained by Hawk and Gies⁹ in which there was loss of nitrogen following severe or repeated hemorrhages.

nitrogen after bleeding may be due to the “washing out” effect of the large amount of water consumed. The permanent occlusion of such

9. Hawk, P. B., and Gies, W. J.: The Influence of External Hemorrhage on Chemical Changes in the Organism, with Particular Reference to Proteid Catabolism, *Am. J. Physiol.* **11**:171, 1904.

large arteries as the femoral artery might be expected to lead to death of tissue and therefore actual catabolism of protein. Extensive and repeated hemorrhages may influence the dog's general metabolism adversely, while moderate hemorrhages would not. Although the metabolism of all three of the animals remained permanently lower after the bleeding, the following conclusion seems justifiable: During

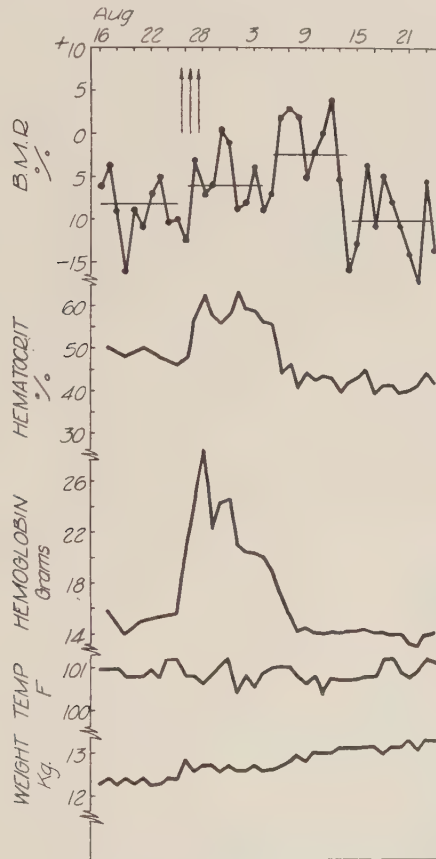


Chart 5 (dog 1).—Blood transfusions were given on August 25, 26 and 27. The amounts were 275, 190 and 155 cc., respectively, and in each instance 50 cc. of 2.5 per cent sodium citrate was used as an anticoagulant.

recovery from acute hemorrhagic anemia of moderate degree in dogs there is a decrease in oxygen consumption which is comparable with that seen in patients with pernicious anemia during the recovery which is induced by liver extract.

Transfusion of Whole Blood.—Dogs 1 and 4 were given transfusions of compatible whole blood in quantities equivalent to about

50 cc. per kilogram of body weight (charts 5, 6, 7 and 8B). Transfusions were given to dog 1 on three successive days and were begun forty days after the previous experiment with bleeding. The weight, blood count and oxygen consumption had reached constant levels before the transfusions were given. Dog 4 had not been bled previously, and neither dog had been given a transfusion previously.

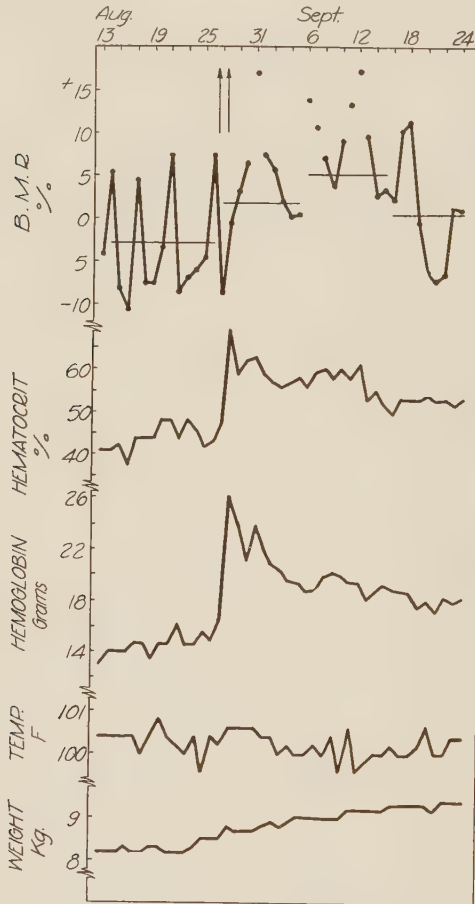


Chart 6 (dog 4).—Transfusions were made on Aug. 26 and 27, 1931. The amounts of blood given were 180 and 245 cc. Several determinations of basal metabolism were considered to be unsatisfactory because the dog moved. These rates are indicated by dots but are not connected by solid lines and were not used in computing averages.

The responses in the two dogs were parallel. There was an immediate increase in hemoglobin and hematocrit values and in erythrocyte counts. The hematocrit values remained fairly constant for ten days in dog 1 and for sixteen days in dog 4. The hemoglobin values seemed

to fall off gradually in both dogs, but the erythrocyte counts paralleled the hematocrit values rather well. Both dogs showed a greater loss of blood during the second period of ten days after transfusion than during the first period of ten days.

The basal metabolic rate increased as the volume of corpuscles decreased. For the first ten days after transfusion the basal metabolic rate was slightly above the control average, 2.3 per cent in dog 1 and

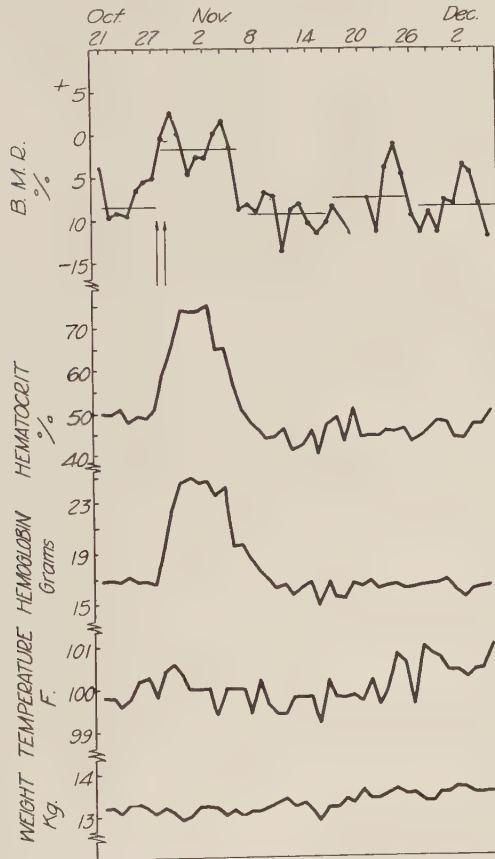


Chart 7 (dog 2).—Two hundred and sixty cubic centimeters of blood was given on October 27 and 330 cc. on October 28.

4.6 per cent in dog 4. In the second period of ten days the average basal metabolism was 6 per cent above the control in dog 1 and 8 per cent above the control in dog 4. In each dog, the blood count and the oxygen consumption returned approximately to normal during the third period of ten days after transfusion. It is admitted that the differences in basal metabolic rates in these experiments are slight, especially in view of the many variable factors influencing the determination of the

oxygen consumption by the method employed. However, the orderliness and parallelism of the changes in the two animals and especially the correlation between the oxygen consumption and the loss of blood are striking enough to appear significant. Changes in body temperature were slight and bore no apparent relation to the changes in oxygen consumption.

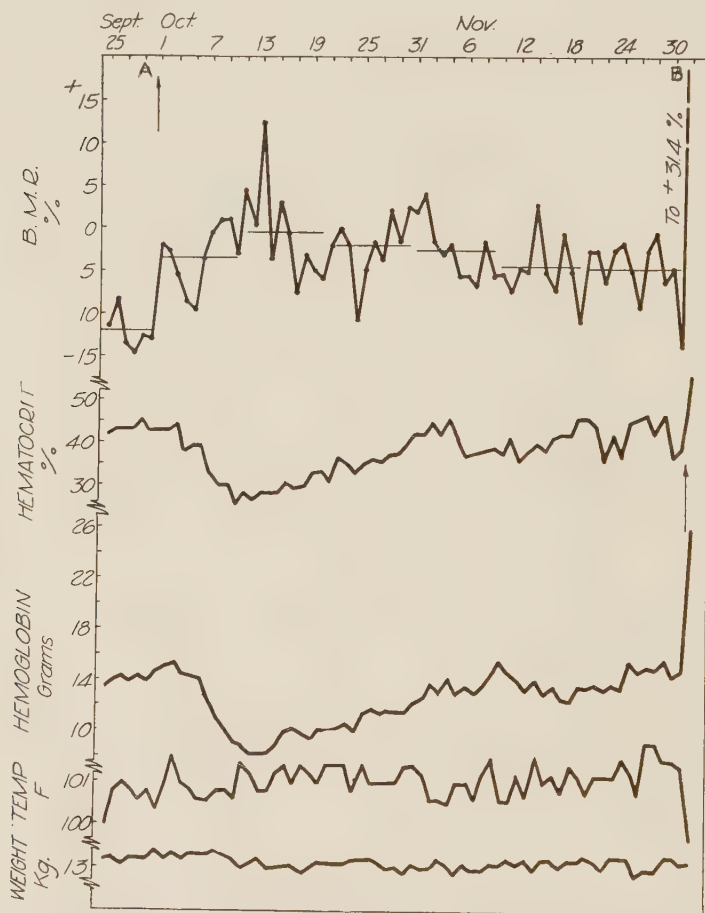


Chart 8 (dog 1).—A, 200 cc. of citrated plasma was given intravenously on September 30. B, 375 cc. of whole blood was given intravenously on Dec. 1, 1931. The dog died on December 2.

A similar experiment was performed in the case of dog 2, but conditions were different in one regard. On Sept. 1, 1931, dog 2 received subcutaneous injections of the plasma and hemolyzed corpuscles from 275 cc. of compatible blood. On September 30 a further injection of 265 cc. of citrated plasma was made intravenously. On October 27 and

28, dog 2 received transfusions totaling 590 cc. of whole blood from a different but compatible donor. This transfusion led to the usual immediate increase in the hemoglobin, volume of corpuscles and erythrocyte count, but loss of blood set in on the fifth day instead of after from ten to sixteen days, as had been the case in dogs which had not previously received transfusions. This earlier loss of blood was associated with an earlier increase in the basal metabolic rate. The average basal metabolic rate for the ten days following transfusion was 5.2 per

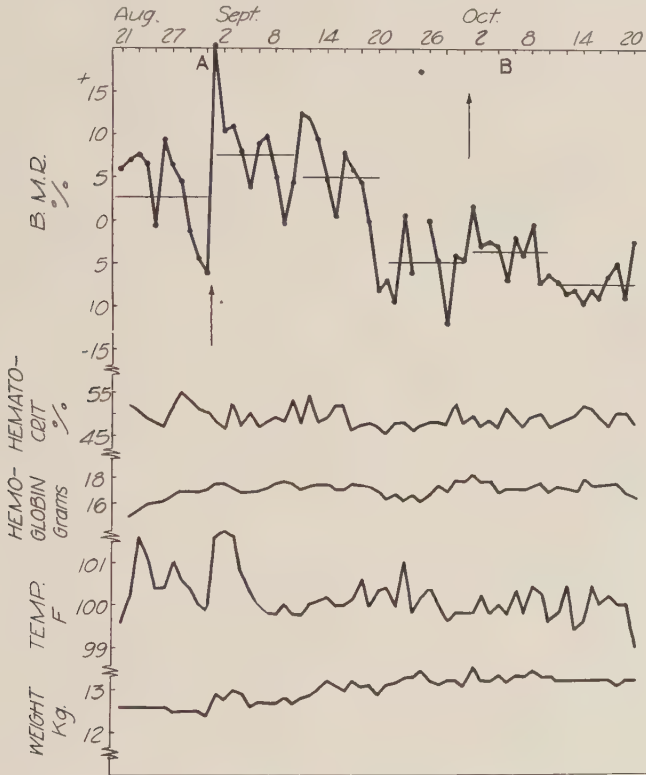


Chart 9 (dog 2).—A, the plasma and hemolyzed corpuscles from 275 cc. of blood were injected subcutaneously on September 1. B, 265 cc. of citrated plasma was administered intravenously on September 30.

cent above the control average. The average of the results of the determinations made during the second period of ten days showed that the gaseous metabolism was 2.4 per cent below the control average. The data in this experiment lend support to the assumption that there is a causal relationship between the loss of blood and the increase in oxygen consumption.

In an effort to study this process in a more accentuated form, dog 1 was given a transfusion of 375 cc. on Dec. 1, 1931. Previous trans-

fusions in the case of this dog included intravenous injections of whole blood in amounts of 275, 190 and 155 cc. on Aug. 25, 26 and 27, 1931, and an injection of 200 cc. of citrated plasma on September 30. All of the transfusions were made from the same donor, the blood of which was originally compatible.

The basal metabolism, which during the control period had averaged 4.5 per cent below normal, increased to 31.4 per cent above normal on the day after the transfusion. With this rise in the gaseous metabo-

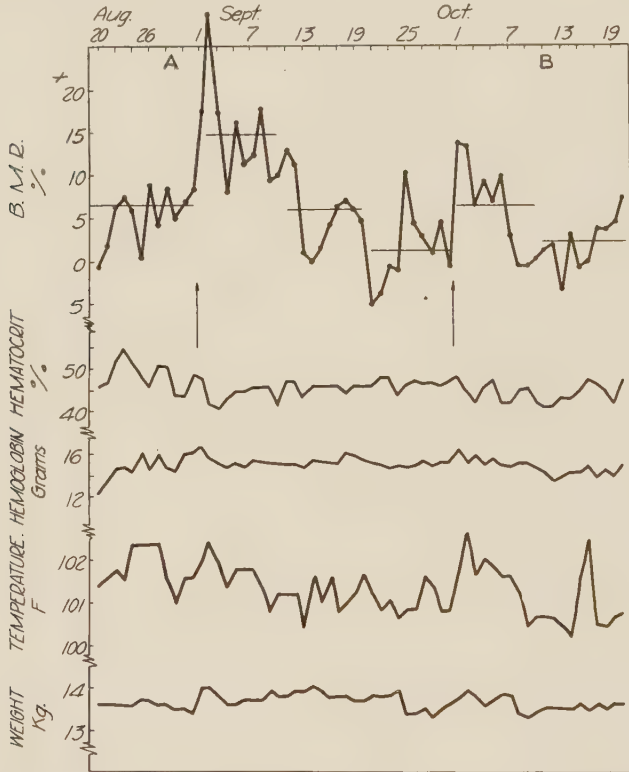


Chart 10 (dog 3).—*A*, the plasma and hemolyzed corpuscles from 325 cc. of blood were injected subcutaneously on September 1. *B*, the hemolyzed corpuscles from 320 cc. of blood were given subcutaneously on September 20.

lism the temperature dropped from 101.5 to 99.8 F. Later in the day the dog died. Necropsy revealed intense congestion of the spleen, liver and lymph nodes, with dark blood in the gastro-enteric tract and hemorrhages into most of the mucous surfaces. Paresis of the caudal half of the body, which appeared soon after the transfusion, was unexplained at necropsy.

Hemolyzed Corpuscles and Plasma.—In order to speed up the catabolism of the hemoglobin of transfused blood, hemolyzed erythro-

cytes were injected into two dogs (charts 9 *A*, 10 *A* and 11 *A*). The corpuscles were hemolyzed in distilled water, and plasma and hemolyzed corpuscles were injected subcutaneously. Dog 2 received 275 cc. of whole blood so treated, and dog 3 was given the plasma and hemolyzed corpuscles from 325 cc. of blood.

There was a prompt outpouring of blood pigments in the urine. The urine was black and gave positive qualitative tests for bile pigments and

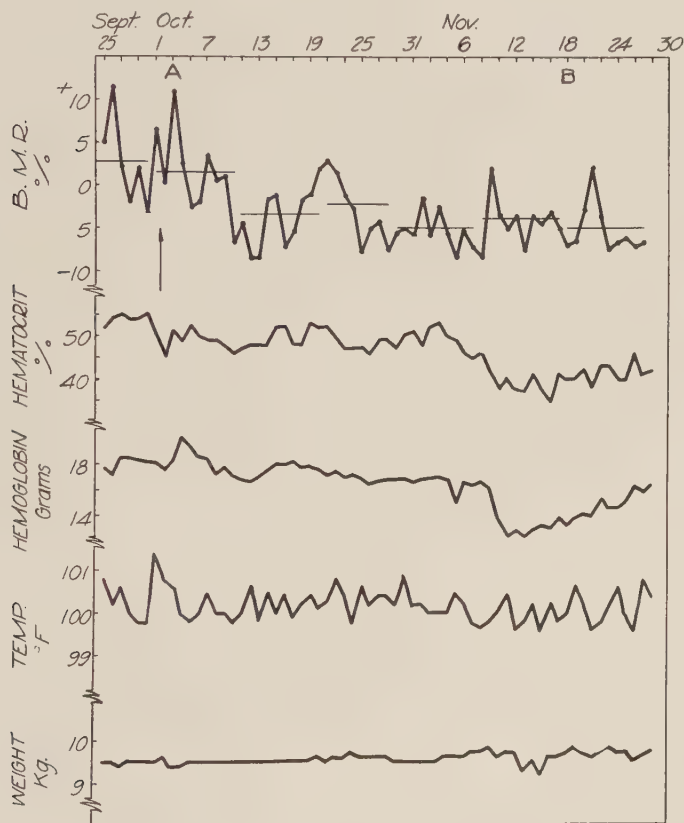


Chart 11 (dog 4).—*A*, the hemolyzed corpuscles from 275 cc. of blood were given on September 30. *B*, from October 28 to November 15 almost daily injections of distilled water were made intravenously. Thirty cubic centimeters was injected daily for ten days, after which injections of the following amounts were made each day: 100, 150, 200, 200, 300, 300, 0.0 and 300 cc. Twice the basal metabolic rate was determined at hourly intervals after injections of 300 cc. (see text). Hemoglobin appeared in the urine after seven of the injections of distilled water.

for hemoglobin. There was also rather marked proteinuria. There was a slight increase in temperature, but in neither case did the temperature exceed the high point in the control period. Following the injec-

tions there was an abrupt rise in the oxygen consumption, which was followed by a gradual fall extending over the succeeding twenty days. The number of erythrocytes and the amount of hemoglobin in the recipient's blood were not affected by this procedure. The urine remained dark for several days.

In these, as in the preceding experiments, the increased oxygen consumption coincided with the period of assumed protein catabolism. Nitrogen balances, however, were not determined.

At a later date dogs 3 and 4 received injections of hemolyzed corpuscles without plasma (charts 10 *B* and 11 *A*). The amounts were smaller, hemoglobin from 180 cc. of blood injected into dog 3 and from 275 cc. of blood injected into dog 4. The results were similar to those in the preceding experiments except that the increase in oxygen consumption was less marked in spite of the fact that the increase in temperature was greater.

An attempt was made to study the effects of *in vivo* hemolysis by distilled water in dog 4 (chart 11 *B*). Distilled water was injected intravenously in amounts varying from 30 to 300 cc. Hemoglobin appeared in the urine on seven occasions, and there was a loss of about 5 Gm. of hemoglobin per hundred cubic centimeters of blood during a period of nine days. However, the oxygen consumption as determined twenty-two hours after the injections remained normal. On two days determinations made at hourly intervals after the injections showed maximum increases of 14 and 69 per cent. On the second day the increase lasted at least ten hours, but there were a chill and an elevated temperature which invalidated the result. On the first day, which presents no doubt the more accurate picture of the changes, the control basal metabolic rate was 3.5 per cent below normal. After the rapid injection of 300 cc. of warm distilled water the rates at hourly intervals were as follows: plus 10.4, plus 9.4, plus 8.7, plus 8.9, plus 2.3 and plus 6.4 per cent. Here, as in the preceding experiments, hemoglobinuria was accompanied by proteinuria. It is recognized, of course, that the nitrogen appearing in the urine as protein or amino-acids must be deducted from the total nitrogen in order to estimate catabolized protein nitrogen.

Plasma Administered Intravenously.—Citratd plasma was injected intravenously in dogs 1 and 2 (charts 8 *A* and 9 *B*). The results were in part unexpected. Dog 2 received 265 cc. of citratd plasma on Sept. 30, 1931. On the day following the injection, the oxygen consumption was about 6 per cent greater than on the day preceding the transfusion, while the average for the ten days following transfusion was about 1 per cent higher than the control average. The hemoglobin, hematocrit value and erythrocyte count were unaffected by this pro-

cedure. The dog had had only one previous injection of plasma and hemolyzed corpuscles from the same donor.

The case of dog 1 was different (chart 8 *A*). Intravenous injection of 200 cc. of citrated plasma was made as in dog 2. The basal metabolic rate, which was 13 per cent below normal on the day before the injection, was only 2 per cent below normal on the following day. The average oxygen consumption for the first ten days after the injection of plasma was 8.5 per cent above the control level, while the average for the second period of ten days was 11.5 per cent above the control level. This increase in oxygen consumption was associated with a loss of hemoglobin from about 15 to about 8 Gm. per hundred cubic centimeters of blood and a corresponding decrease in hematocrit value and erythrocyte count. Unfortunately, estimations of the urobilin and urobilinogen content of the stools and urine and nitrogen balances were not made, so that it is not possible to attribute the loss of blood definitely to hemolysis. Recovery of the blood elements was complete, however, a circumstance which makes it unlikely that the loss of blood was due to aplasia of the bone marrow.

In these experiments it appears that destruction of blood was produced in one animal and not in the other. It further appears that a rather nicely controlled experiment showing the effect of the destruction of blood on the total oxygen consumption has been unwittingly accomplished. The results are in complete accord with the general thesis, i. e., that the oxygen consumption is increased by increased endogenous protein catabolism.

Diabetes Caused by the Administration of Phlorhizin.—Phlorhizin diabetes seemed to present the most ideal situation for putting the previously suggested general thesis to a crucial test (charts 12, 13 and 14). In the phlorhizinized animal endogenous protein catabolism proceeds at a greater rate than in any other nonfatal condition that I know. As the effects of phlorhizin disappear, the animal is left in good health, except that his protein is much depleted. Such a condition should therefore be ideal for the study of the effects of endogenous protein catabolism as well as of the effects of marked retention of nitrogen in the laboratory animals. It was demonstrated by Rubner¹⁰ that the specific dynamic effect of catabolized protein of endogenous origin could account for the marked increase in oxygen consumed by the fasting phlorhizinized dog. From such calculations Lusk concluded that protein of endogenous origin exerts the same specific dynamic effect as a similar amount of exogenous protein. To my knowledge, no one has previously studied the oxygen consumption of animals during the period of nitrogen storage in the recovery from diabetes due to the administration of phlorhizin.

10. Rubner, M.: *Die Gesetze des Energie verbruchs bei der Ernährung*, Leipzig, Franz Deuticke, 1902, p. 370; quoted by Lusk,⁶ p. 282.

Dog 2 was given the regular diet up to and including Dec. 15, 1931. After the determination of the basal metabolism on December 16, 1 Gm. of phlorhizin was injected subcutaneously. No more food was given for eight days, during which there was evidence of marked endogenous protein catabolism. The D:N ratio reached only 1.93, so that the animal was not completely phlorhizinized. On December 24, food, consisting of 225 Gm. of bread, 250 cc. of milk and 20 Gm. of bone meal or 4.6 Gm. of nitrogen, was allowed. The oxygen con-

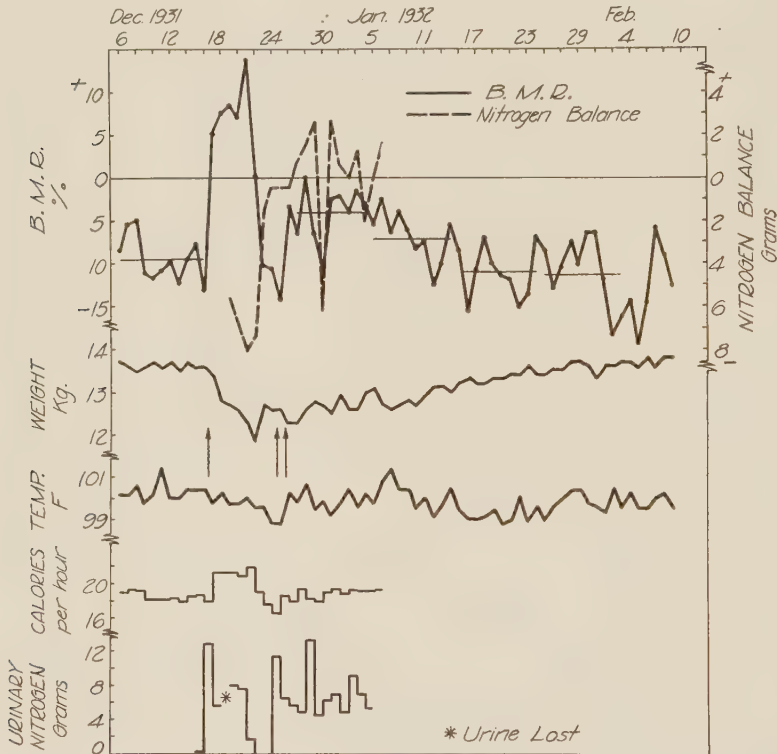


Chart 12 (dog 2).—The first arrow (from left to right) indicates the beginning of the fast and the administration of 1 Gm. of phlorhizin subcutaneously on December 16. The second arrow indicates when food was first given, on December 24, 225 Gm. of bread, 250 cc. of milk and about 20 Gm. of bone meal. The third arrow indicates the return to the normal diet. The nitrogen balance is not accurate, since fecal nitrogen and the nitrogen lost in hair were not determined. The irregularity in the output of urinary nitrogen was caused by the fact that the dog had been conditioned not to urinate in a cage.

sumption, which had been falling to about parallel with the fall in nitrogen catabolism, continued to fall with the ingestion of the aforementioned amount of protein. From December 25 to the end of the experiment the animal was given its regular diet of 150 Gm. of chopped

raw beef lung, 225 Gm. of bread, 250 cc. of milk and 20 Gm. of bone meal, a total of 6.8 Gm. of nitrogen daily. After the return to a normal diet there was an immediate increase in the amount of urinary nitrogen with a similar increase in gaseous metabolism (chart 12). Nitrogen-retention was not constant or marked. It seemed evident that the animal was continuing to break down protein instead of storing it. This

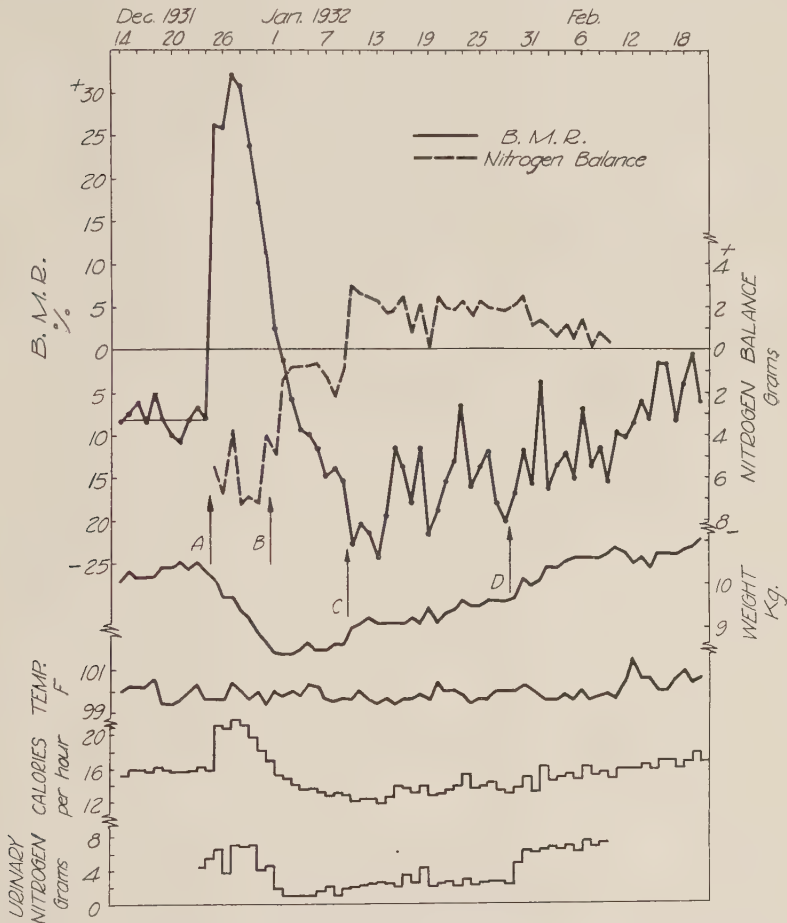


Chart 13 (dog 4).—Arrows indicate the following: *A*, subcutaneous injection of 1 Gm. of phlorhizin and the beginning of the fast; *B*, from 50 to 200 Gm. of sugar daily (total 1,050 Gm.); *C*, 200 Gm. of bread, 20 cc. of milk and about 20 Gm. of bone meal daily; *D*, 200 Gm. of meat was added to the diet. Again the nitrogen balance did not include nitrogen lost in the feces or hair.

may have been due to the fact that the stores of glycogen were empty when realimentation with a mixed diet occurred. An attempt was made to control this factor in the succeeding experiments.

In the case of dog 4 (chart 13) the procedure was the same, except that on the seventh day after phlorhizin was administered, 50 Gm. of dextrose was given by mouth. On the six succeeding days the animal received 100 Gm. of dextrose daily and then 200 Gm. daily for two days. During this time the gaseous metabolism and the urinary nitrogen continued to decrease. The average basal metabolic rate during the

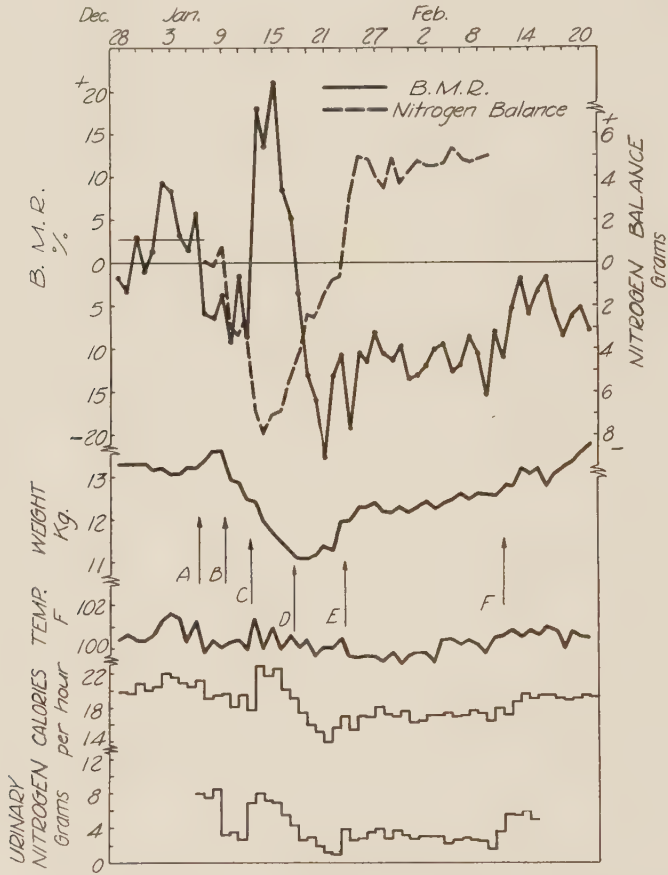


Chart 14 (dog 3).—The arrows indicate the following: *A*, the dog was moved from regular animal quarters to the cage in the metabolism room; *B*, the fast was begun; *C*, 1 Gm. of phlorhizin was given; *D*, from 100 to 300 Gm. of sugar (total, 2,000 Gm.) was given; *E*, 300 Gm. of milk and 20 Gm. of bone meal were given daily; *F*, 200 Gm. of meat was added.

control period was 8.1 per cent below normal. During the period of increased protein catabolism after phlorhizin the metabolic rate reached 32.1 per cent above normal, but the oxygen consumption fell to 15.5 per cent below normal, as endogenous protein was spared by the feeding of sugar. On December 9 the diet was changed to 200 Gm. of bread,

250 cc. of milk and 20 Gm. of bone meal or 4.56 Gm. of nitrogen a day. This diet was attended by marked and consistent nitrogen retention and a further fall in the oxygen consumption to 24.4 per cent below normal. This second fall in gaseous metabolism which occurred when the animal was receiving a diet low in proteins is not understood, since the actual amount of nitrogen in the urine was greater than it had been on the preceding days when only carbohydrate was contained in the diet. Special attention is directed to the fact that in this animal the basal metabolic rate fell from plus 32.1 to minus 24.4 per cent, a total excursion of 56.5 per cent, without change in temperature. The decrease from plus 32.1 to minus 15.5 per cent appeared to be caused entirely by a drop in the catabolism of endogenous protein.

In dog 3 the procedure and results were almost exact duplicates of those in dog 4 (chart 14).

The following explanation is offered for the results in all of the experiments described:

Endogenous protein, when catabolized, exerts the same specific dynamic effect as would a similar amount of ingested protein. The difference is that the effect of exogenous protein can be avoided to a great extent by making determinations of the basal metabolic rate only in the postabsorptive period. Endogenous protein catabolism, on the other hand, is a continuous process, and some of the amino-acids so derived exert their effect throughout the day.

The explanation for the lowered gaseous metabolism during the storage of nitrogen is not quite so clear but no doubt depends in part at least on the fact that stored amino-acids exert no specific dynamic effect. From numerous data it seems probable, though not proved, that the lowered oxygen consumption during the storage of nitrogen is due not so much to the storage of nitrogen per se as to a lowered total endogenous catabolism of protein during the postabsorptive period when storage is in progress.

Numerous data in the literature support this thesis directly or indirectly. Only a few will be mentioned. Rubner¹¹ and Hoobler¹² independently showed that stored protein does not exert a specific dynamic effect. Lee and Gagnon¹³ showed that rats growing rapidly under the influence of the growth-promoting hormone from the anterior pituitary lobe have a lowered basal metabolism as referred to weight or surface area. The realimentation following a fast is not attended

11. Rubner,¹⁰ p. 246, quoted by Lusk,⁶ p. 297.

12. Hoobler, B. R.: The Protein Need of Infants, *Am. J. Dis. Child.* **10**:153 (Sept.) 1915.

13. Lee, M. O., and Gagnon, J.: Effect of Growth Promoting Extracts of the Anterior Pituitary on Basal Gaseous Metabolism in Rats, *Proc. Soc. Exper. Biol. & Med.* **28**:16, 1930.

by an immediate return of the oxygen consumption to the prefasting level (Kleitman¹⁴ and Anderson and Lusk¹⁵).

Evidence that increased protein catabolism is associated with an augmented gaseous metabolism is also to be found in the literature. Du Bois¹⁶ and his co-workers demonstrated that the increased oxygen consumption in febrile diseases was more or less proportional to the degree of endogenous protein catabolism which was present. For example, for a given elevation in temperature typhoid fever usually presented a greater protein catabolism and a greater oxygen consumption than did pulmonary tuberculosis. Similarly, increases in endogenous protein catabolism¹⁷ and in oxygen consumption occur coincidentally in the first few days of a fast and possibly during the premortem rise in nitrogen excretion. Wilder, Boothby and Beeler¹⁸ demonstrated a definite relationship in diabetes between the amount of protein in the diet and the basal metabolism. Heinbecker¹⁹ found that the gaseous metabolism of Eskimos was considerably higher than that of people living in the temperate zone. At the Russel Sage Institute²⁰ two Arctic explorers were kept on a diet of meat for a year. After six weeks the basal metabolism had increased slightly, but at the end of a year it was back to the level which had been observed for a mixed diet. There was a suggestion that the specific dynamic action of protein increased after long periods on diets of meat. In most instances the diets were much higher in fat than in protein. There is a possibility, of course, that the presence of acetone influenced the results obtained by the investigators, who studied subjects receiving diets high in fat and relatively high in protein but without carbohydrate.

SUMMARY

1. In pernicious anemia the basal metabolic rate decreases during periods of rapid regeneration of blood.
2. In leukemia the total oxygen consumption is increased during the increased catabolism of endogenous protein induced by irradiation.

14. Kleitman, N.: Basal Metabolism in Prolonged Fasting in Man, *Am. J. Physiol.* **77**:233, 1926.

15. Anderson, R. J., and Lusk, G.: Animal Calorimetry: The Interrelation Between Diet and Body Condition and the Energy Production During Mechanical Work, *J. Biol. Chem.* **32**:421, 1917.

16. Du Bois, E. F.: The Basal Metabolism in Fever, *J. A. M. A.* **77**:352 (July) 1921.

17. Lusk,⁹ p. 78.

18. Wilder, R. M.; Boothby, W. M., and Beeler, C.: Studies of the Metabolism of Diabetes, *J. Biol. Chem.* **51**:311, 1922.

19. Heinbecker, P.: Studies on the Metabolism of Eskimos, *J. Biol. Chem.* **80**:461, 1928.

20. McClellan, W. S.; Spencer, H. J., and Falk, E. A.: Clinical Calorimetry: XLVII. Prolonged Meat Diets with a Study of the Respiratory Metabolism, *J. Biol. Chem.* **93**:419, 1931.

3. The destruction of erythrocytes by phenylhydrazine in polycythemia vera is attended by an increase in gaseous metabolism.

4. In dogs the oxygen consumption is decreased during recovery from moderately severe acute hemorrhagic anemia.

5. Compatible blood transfused into dogs is lost from the peripheral circulation at varying rates. The rapidity of loss of blood seems to be increased in successive transfusions. The basal metabolic rate of the dog is increased during the period of loss of blood.

6. The pigment from hemolyzed blood is rapidly excreted by dogs, and again the catabolic process is attended by an increase in gaseous metabolism. The increased metabolism occurs whether the blood is hemolyzed in vivo or is obtained from another animal.

7. The injection of citrated plasma into dogs is sometimes attended by a delayed loss of blood in the recipient, and this loss of blood appears to cause an increase in gaseous metabolism.

8. Phlorhizin diabetes in the dog is associated with a great increase in endogenous protein catabolism which is followed under certain conditions by marked storage of nitrogen. The oxygen consumption in the phlorhizinized animal parallels the endogenous protein catabolism.

